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COMPARATIVE BIOMONITORING OF CERTAIN DUMRE LAKES TENABLE ON CELL CYCLE

ABSTRACT

Dumre lakes with karst origin are mostly supplied by the rain water considered, until 30–40 years ago, as drinkable hydric resource. Nowadays, the loss of forests and soil degradation around them, likewise the intense agricultural practice and a number of anthropogenic activities carried out close to the lakes are very complex processes which may facilitate the eutrophication process. We evaluated the cytotoxic parameters as biomarkers of pollution from some lakes of Dumre. Three dry bulbs of *Allium cepa* were germinated in sample water, at room temperature. Controls were treated as well with tap water for the same period of time. The root tips were stained with aceto-orceine and then squashed and observed microscopically. The cytotoxity induced was compared with the value for the concomitant negative control using t-test. Only Belshi lake (23.7-52%) caused a significant reduction in the mitotic index compared with the negative control, which was indicative of lethal effects on test organism. Merhoja lake had the lowest mitodepressive value (43-13%). Comparing the phase indices, the separate phase durations were higher for the telophase index in samples of Merhoja and Seferan lakes showing a prompt cell division rate in that period. Decrease in the cell division process results from the effects of cytotoxity environmental chemicals which have the potential to cause adverse human health and environmental impact. We must consider necessary biomonitoring these lakes sited in a partially protected area.

Keywords: *cytotoxity, Allium cepa, mitotic index, environmental impact.*

INTRODUCTION

The lakes of Dumre are located in central Albania (Fig.1), south of Elbasan, in a zone that extends between Shkumbini and Devolli valleys. More than 100 karst lakes with highly variable lengths and depths cover an overall area of 660 ha, which corresponds to 3% of the entire outcropping area of evaporites in our coun-

try (Parise *et al.*, 2004). Four lakes are longer than 1 km, with lake Seferan being the longest (over 2 km), whereas the deepest is lake Merhoja, which reaches 61 m in depth (Table 1). Most of the lakes are deeper than 10 m, and cover areas between 3 and 100 ha. The majority of the lakes are circular in shape, but are present elliptical lakes (such as lake Deges) and also irregular shapes (Kristo, 1994).

Fig. 1. Geographical position of Elbasan district.

Dumre lakes are mostly supplied by the rain water and do not have inflows or outflows, hence, their water level vary seriously during the year. Before 1950, the region was used as pasture but afterwards it changed totally to agriculture development, reducing in this way the surface covered by wood and on the other hand raising the sediment production (Qiriazi *et al*., 1999).

Besides agriculture activities carried out near the lake boundaries using intensively herbicides and pesticides, uncontrolled urban sewage effluent would notedly contribute to incessant deterioration of standart water quality (Vrijheid, 2000; Palmer *et al*., 2005).

Table 1. Morphometry of the main karst lakes in the evaporite area of Dumre.

Lake name	Area (ha)	Average depth (m)	Max depth (m)	Length (km)	Width (km)	Lake perimeter (km)
Çestija	98.6	4.4	11.1	1.2	0.72	6.3
Seferan	87.5	4.5	20.8	2.05	0.5	5.9
Merhoja	65.5	17.5	61	1.42	0.73	3.6
Deges	37.4	–	17.9	1.6	0.3	3.8
Paraska	27.4	5.4	–	0.78	0.5	2.2
Belshi	26.9	4	13.1	0.76	0.4	0.4

To indicate the level of pollution we used mitotic index as an eligible measure of cytotoxicity for all living organisms (Smaka-Kinel *et al*., 1996). The reduced rate of mitotic index can define the level of cytotoxicity. A decrease of mitotic index below 50% usually has lethal effects (Panda and Sahu, 1985). If mitotic index decreases below 22% of control, that it causes sub-lethal effects on test organism (Antonsie-Wiez, 1990). The presence of potential cytotoxic and genotoxic substances in lake water test of Belshi, Seferani, Merhoja and Gjoli i gjatë was measured using *Allium cepa* testing procedure. The *Allium* test was selected among other bioassays as a common test with many advantages as (Leme and

Marin-Morales, 2009; Firbas and Amon, 2013) a short-term one with low cost, ease to handle, stable chromosome conditions for the study of chromosome damage or disturbance of cell division and as a sensitive test showing good correlation to other test systems.

The aim of this study therefore, was to evaluate the cytotoxic potential of some important Dumre lakes selected by utilizing root meristem cells of *A. cepa*.

MATERIALS AND METHODS

Samples from Belshi, Merhoja, Seferani and Gjoli i gjatë lakes were taken during October 2016 (Fig. 2). Onion bulbs were carefully pruned at the base leaving the ring of root primordial intact (Fiskesjo, 1985). Each one was placed on 250 ml beakers filled with the samples water with the base of the onion bulbs touching the surface of the liquid. Three onion bulbs were arranged for each pattern of the selected samples at room temperature. As a control was used tap water placed on beakers with the same conditions as cases.

Measure of the length and number of roots was carried out at 24, 48, 72 and 96 hours, twice a day (Fig 3.). Calculations of the mean and inhibition of root growth were compared with the results of the control samples. For cytogenetic investiga-

a) b)

Fig. 2. a) View of lake Belshi wherein noticed houses situated close to the water. b) Part of lake Merhoja.

tions, after the fourth day root tips from each samples were cut and slides were prepared for each variant using the squash technique, stained with aceto-orcein and refrigerated for 48 h. After fixing with acetic acid, with a gentle tapping of the cover slip the cells were spread in a lineup and the excess stain was removed using a filter paper.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total cells counted}} \times 100$$

Diverse mitotic stages were counted in at least 100 cells in order to determine the mitotic index. The microscopic examination was performed under a compound microscope at 1000 magnifications, equipped with a digital camera.

Fig. 3. Root growth at the end of the experiment.

The means, with 95% confidence limits and the standard errors for results of the root inhibition and chromosome aberrations were calculated for each sample of the waters. Data were expressed as Mean Differences between the control and the different samples of the lake water were analyzed by means of the Student's unpaired t-test.

RESULTS AND DISCUSSIONS

The application of bioassays for estimating the toxicity hazard discharged to an aquatic environment is a superior way of predicting the risk than chemical analysis. Chemicals in the water form a complex mixture which is difficult to assess the influence posed using standard chemical identification techniques (Calabrese and Blain, 2005). For toxicity test is used *Allium* test as a common and sensitive one which offers an accurate evaluation of different sub-lethal and lethal levels producing relevant results with others (Fiskesjo, 1995).

In our study, a time dependent root length inhibition was observed with respect to control, in all the cases. During the initial exposure period the root growth was not affected by the possible toxic elements present in the water doses collected in autumn season (Table 2). The mean root length ranged from 3.4-3.9 cm in root cells treated with Belshi water to 6.7 cm in the root cells of the control. With increase in the time of exposure the difference compared to control was significantly augmented, where as Belshi N samples inhibited more the root growth with 49% indicating a higher toxicity of the water lake. The lowest root growth inhibition value with respect to control was observed up to 4% in lake Merhoja dependent by the weak presence of toxic elements.

Mitosis was observed to be normal in the root cells obtained from the control (tap water) and treatments. Chromosome gauges at metaphase and other stages pointed out normal cell division without any aberration, expect a scurvy number of

cases. Table 3 shows the data on the effect of the sample water lakes in the mitotic activities of the cells of *Allium cepa*.

Table 2: Root growth inhibition values of Allium bulbs after 4 days treated with certain lakes water.

Samples	Mean root length (in cm)	% Root Growth Inhibition
Tap water	6.7	
Seferan	4.4	34
Merhoje	6.4	4
Gjoli i gjate	5.1	24
Belshi E	3.9	41
Belshi N	3.4	49

The mitotic index in the treated roots were lower than the control (Table 3). The mitotic index values ranged between 23.7% in the roots treated with lake Belshi water obtained from the N part, and 43% in the roots treated with lake Merhoja water. Whereas the mitotic index value of the control was 49.53%. There was an obvious difference of decrease in the total percent number of divided cells. From the aforesaid results, the lower mitotic index than the control indicated that the lake Belshi water samples are more mito-depressants than the others.

Parameters such as root growth, frequencies of mitosis and abnormal cells can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of substances (Monte Egito *et al.,* 2007; Thomas *et al.*, 2009; Schuch *et al.*, 2012; Firbas and Amon, 2013). Root growth restriction in *Allium cepa*, indicates restriction of growth and cytotoxicity, which are associated with suppression of mitotic activity. The current study registered low mitotic index values for treated onion cells, which showed that cell divisions were depressed in the samples root cells. This is in accordance with the findings of Sang *et al.* (2006) and Kjeldsen *et al.* (2002) who presented lower mitotic index values in treated root cells compared with the controls.

Table 3: Mitotic activities of *Allium cepa* root cells treated with certain lakes water.

Samples	No. of divided cells	Total cells	Mitotic Index
Tap water	181	404	49
Seferan	226	683	33
Merhoje	426	983	43
Gjoli i gjate	93	293	31
Belshi east	106	396	26.26
Belshi north	55	223	23.7

A reduction in the mitotic index was probably due to the blocking of G1, suppressing cells from DNA synthesis or the blocking of G2, preventing cells from entering mitosis (Amin, 2002; Li *et al.*, 2008). However, the occurrences of higher number of

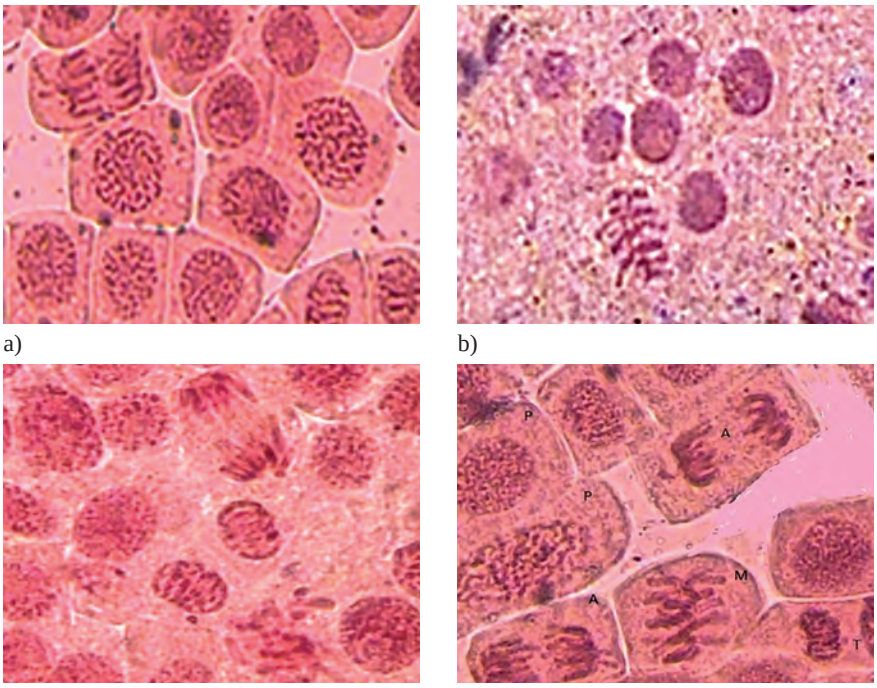

Figure 2. *Allium cepa* meristematic cell. (a) Anaphase cell with chromosome break in Belshi E samples. Normal interphase cell. (b) Normal metaphase cell of Merhoja sample. (c) Anaphase cell with chromosome loss and C-mitosis. (d) Normal prophase, metaphase, anaphase and telophase cells.

metaphase and metaphase/prophase in root cells of the Belshi N bulbs indicate that majority of the dividing cells are in the prophase stage (Table 4). This indicates that after the mitotic suppression, the surviving cells started to divide again, but didnot pass mitosis stage. The separate phase durations were higher for the telophase and anaphase index in samples of Merhoja and Seferan lakes showing a termination of cell division rate in that period (Table 4).

It should be noted the available studies focused on an ecological and ecotoxicological perspective for these lakes. In the study of Sula and Aliko (2017) was concluded the presence of a number of histological changes in the liver of the fish, *Carassius carassius* present in the same lakes studied here. High values for biochemical oxygen demand after 5 days, and coliforms were found mainly in lake Belshi and with variation in some parts of lakes Seferani and Merhoja (Mali, 2010) which were responsible for the poor level of classification water quality.

Table 4. Phase index of *Allium cepa* root cells treated with sample water.

Samples	Prophase	in%	Metaphase	in %	Anaphase	n%	Telophase	in %
Tap water	156	86	7	3.8	3	1.6	15	8.2
Seferan	198	87.6	4	1.76	5	2.2	19	8.4
Merhoje	367	91	7	1.6	10	2.3	32	7.51
Gjoli i gjate	39	90	1	1.07	2	2.15	6	6.45
Belshi E	96	90.5	2	1.8	2	1.8	6	5.6
Belshi N	47	85.5	4	7.27	1	1.8	3	5.45

In Slovenia, from 1990 until 2011, was practiced more than 1230 *Allium* tests on 390 localities with high tendency to increase its use on 200 lakes of this country (Firbas, 2011). Whereas, no systematic evidence are available to control the type and amount of polluting substances, and, to our knowledge, no review of the quality of lakes water has started. About 100 lakes and a number of water habitants in Dumre represent sensitive ecosystems, which needs to be biomonitored for the quality of water, in order to keep these ecosystems clean and healthy.

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